Effect of dietary protein concentration and degradability on response to rumen-protected methionine in lactating dairy cows¹

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ABSTRACT

An incomplete 8 × 8 Latin square trial (4-wk periods; 12 wk total) using 32 multiparous and 16 primiparous Holstein cows was conducted to assess the production response to crude protein (CP), digestible rumen-undegraded protein (RUP), and rumen-protected Met (RPM; fed as Mepron; Degussa Corp., Kennesaw, GA). Diets contained [dry matter (DM) basis] 21% alfalfa silage, 34% corn silage, 22 to 26% high-moisture corn, 10 to 14% soybean meal, 4% soyhulls, 2% added fat, 1.3% minerals and vitamins, and 27 to 28% neutral detergent fiber. Treatments were a 2 × 2 × 2 factorial arrangement of the following main effects: 15.8 or 17.1% dietary CP, with or without supplemental rumen-undegraded protein (RUP) from expeller soybean meal, and 0 or 9 g of RPM/d. None of the 2- or 3-way interactions was significant. Higher dietary CP increased DM intake 1.1 kg/d and yield of milk 1.7 kg/d, 3.5% fat-corrected milk (FCM) 2.2 kg/d, fat 0.10 kg/d, and true protein 0.05 kg/d, and improved apparent N balance and DM and fiber digestibility. However, milk urea N and estimated urinary excretion of urea-N and total-N also increased, and apparent N efficiency (milk-N/N-intake) fell from 33 to 30% when cows consumed higher dietary CP. Positive effects of feeding more RUP were increased feed efficiency and milk fat content plus 1.8 kg/d greater FCM and 0.08 kg/d greater fat, but milk protein content was lower and milk urea N and urinary urea excretion were elevated. Supplementation with RPM increased DM intake 0.7 kg/d and FCM and fat yield by 1.4 and 0.06 kg/d, and tended to increase milk fat content and yield of milk and protein.

Key words: rumen-protected methionine, dietary crude protein, milk yield, nitrogen efficiency

INTRODUCTION

When cows are fed more CP than is needed to meet their requirement for metabolizable AA, the excess N is excreted in the urine and can contribute to environmental pollution. Supplementing diets with RUP or the limiting essential AA may permit a reduction in total dietary CP. Methionine is often first limiting for production in dairy cows fed diets based on legume forages, corn silage, corn grain, and soybean meal (Schwab et al., 1976; Pisulewski et al., 1996; NRC, 2001). Several studies have shown that supplementing these diets with rumen-protected Met (**RPM**) increased milk yield (Schmidt et al., 1999) as well as milk protein content (Armentano et al., 1997; Berthiaume et al., 2006) and yield (Armentano et al., 1997; Rulquin and Delaby, 1997). In one study, we observed that milk and protein yield increased when CP was reduced from 18.3 to 17.3% and to 16.1% when diets were also supplemented with RPM; however, further reduction of dietary CP to 14.8% depressed production of milk, FCM, and fat from that at 16.1% (Broderick et al., 2008).

Leonardi et al. (2003) reported that supplementing with RPM increased milk protein concentration at both 16.1 and 18.8% dietary CP, with no interactions, suggesting that there would be a similar response in protein yield when providing the limiting AA to diets that differ in CP content. However, in the second of our 2 studies (Broderick et al., 2008), RPM did not improve production of milk or milk components when added to diets containing either 16.1 or 17.3% CP, perhaps because cows in that trial consumed about 10% less DM and mobilized body protein, compared with cows in the first study where RPM supplementation allowed the feeding of less CP. Reduced DM intake would reduce MP supply from both microbial synthesis and RUP; thus, differences in response will likely occur with differences in the amount of MP available to support production.

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A follow-up trial was conducted to study the effects on production and nutrient utilization of adding RPM as Mepron (Degussa Corp., Kennesaw, GA) to diets varying in MP supply due to differing levels of CP and RUP. Diets containing 15.8 or 17.1% CP were supplemented with soybean meal RUP and RPM to determine effects on production of milk and milk components.

MATERIALS AND METHODS

At the beginning of the trial, 32 multiparous Holstein cows (mean parity 2.6, SD 0.8), averaging (mean $\pm$ SD) 43 ± 6 kg of milk/d, 128 ± 45 DIM, and 633 ± 46 kg of BW, and 16 primiparous Holstein cows, averaging 39 ± 2 kg milk/d, 132 ± 45 DIM, and 563 ± 59 kg of BW, were blocked by parity and DIM and randomly assigned within squares to treatment sequences in 6 replicated, incomplete 8×8 Latin squares with 3 experimental periods. Periods lasted 28 d and consisted of 14 d for diet adaptation and 14 d for data and sample collection. All cows were injected with bST (500 mg of Posilac, Monsanto, St. Louis, MO) beginning on d 1 of the trial and at 14-d intervals thereafter until completion of the study. Cows were housed in tie stalls and had free access to water throughout the experiment. Care and handling of the animals was conducted as outlined by the guidelines of the University of Wisconsin institutional animal care and use committee.

Diets were fed as TMR and were formulated principally from alfalfa silage, rolled corn silage, rolled high-moisture shelled corn, solvent-extracted soybean meal, expeller soybean meal (Soyplus, West Central Coop, Ralston, IA), soyhulls, plus other ingredients. Diets were fed as a $2 \times 2 \times 2$ arrangement of treatments: 15.8 or 17.1% CP (increased by substituting part of the high-moisture shelled corn with soybean meal), supplemental protein from only solvent-extracted soybean meal or from a mixture of solvent-extracted soybean meal and expeller soybean meal, and 0 or 9 g of RPM/d, added to the diet as 0.062% Mepron (Degussa Corp.), assuming an intake of 24 kg of DM/d and an equivalence of 0.6 g of metabolizable Met/g of Mepron (Berthiaume et al., 2001). The TMR were prepared by blending individual feed ingredients plus a premix composed of ground shelled corn, minerals, vitamins, and Mepron; the premix was added to the TMR at 3.36% of the DM. Diets were offered once daily at 1000 h after orts were collected daily at 0900 h. Amounts of feed offered were adjusted daily to allow refusals equal to 5 to 10% of intake.

Daily samples of approximately 0.5 kg of silages, high-moisture shelled corn, each TMR, and orts were collected, stored at -20°C , and used to make weekly

composites. Weekly samples of the soybean meals, soyhulls, added fat source, and premix also were taken.

Dry matter contents of samples of weekly composites and individual feed samples were determined by drying at 60°C (forced-air oven) for 48 h. The 60°C DM contents of dietary ingredients were used weekly to adjust as-fed compositions of the TMR. Dry matter intake was computed based on the 60°C DM contents of the TMR and orts. These samples were ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA) and analyzed later for total N (Leco FP-2000 Nitrogen Analyzer, Leco Instruments Inc., St. Joseph, MI), absolute DM, ash, and OM (AOAC, 1980), sequentially (Van Soest et al., 1991) for NDF, ADF, and ADIN using heat-stable α -amylase and Na_2SO_3 (Hintz et al., 1995), and for neutral detergent insoluble N (NDIN) without use of Na_2SO_3 (Licitra et al., 1996). For AA determination, samples of feed were predigested with performic acid to stabilize Met and Cys, treated with hydrobromic acid to destroy the performic acid, and then acid-hydrolyzed with 6 N HCl (method 994.12; AOAC, 1997). A separate acid hydrolysis (6 N HCl) and digestion procedure was used for Phe, Tyr, and His because those AA are destroyed during the oxidation process and by reaction with hydrobromic acid. Concentrations of AA were quantified by ion exchange chromatography (Beckman 6300, Beckman Instruments, Palo Alto, CA). Silage extracts were prepared in distilled water from weekly composites according to Muck (1987), pH determined immediately, and extracts were analyzed for ammonia and total free AA (Broderick et al., 2004) using flow-injection (Lachat Quik-Chem 8000 FIA Lachat Instruments, Milwaukee, WI), and for NPN (Muck, 1987; VarioMax CN, Elementar Analysensystem GmbH, Hanau, Germany). Weekly TMR composites were analyzed for indigestible ADF (ADF remaining after 12 d of in situ incubation; Huhtanen et al., 1994). The TMR composites also were analyzed for total fat (method 920.39; AOAC, 1997; Dairyland Laboratories, Arcadia, WI), starch (Hall et al., 1999), and NDIN (Van Soest et al., 1991; Hintz et al., 1995) to compute NFC. Compositions of major feed ingredients are in Table 1 and that of diets are in Table 2.

Cows were milked twice daily at 0600 and 1700 h, and milk yield was recorded at each milking in all experimental periods. Milk samples from a.m. and p.m. milkings were collected on d 17 and 24 of each period and analyzed for fat, true protein, lactose, and SNF by infrared analysis (AgSource, Verona, WI) with a Foss FT6000 (Foss North America Inc., Eden Prairie, MN) using AOAC (1990; method 972.16). For MUN determination, 5 mL of milk samples from both milkings was treated with 5 mL of 25% (wt/vol) TCA. Samples

Table 1. Chemical composition of silages and principal concentrate ingredients¹

Item	AS	CS	HMSC	SSBM	ESBM	Soyhulls
DM, %	42.0	36.0	70.0	89.5	89.8	92.4
CP, % of DM	23.8	7.7	8.1	54.6	48.2	12.2
Ash, % of DM	12.3	3.28	1.23	7.66	6.88	5.36
NDF, % of DM	38.3	39.5	8.6	7.8	17.0	64.0
ADF, % of DM	27.5	23.1	2.1	4.6	7.3	44.8
Hemicellulose, % of DM	10.8	16.4	6.5	3.2	9.7	19.2
NDIN, ² % of total N	13.2	19.3	14.6	7.5	27.6	11.0
ADIN, % of total N	5.4	5.3	3.3	1.1	4.1	5.5
Fraction B3, ³ % of total N	7.9	14.0	11.3	6.4	23.6	5.5
NFC, ⁴ % of DM	21.6	44.4	76.6	25.7	17.6	15.9
Essential AA (g/100 g of CP)						
Arg	2.11	2.48	4.09	7.61	7.34	5.03
His	1.98	1.89	2.81	2.76	2.72	2.47
Ile	4.01	3.20	3.29	4.71	4.67	3.63
Leu	7.12	8.33	11.35	8.09	7.85	6.18
Lys	4.29	2.52	2.93	6.43	5.95	6.10
Met	1.41	1.40	1.89	1.50	1.40	1.15
Cys	0.87	1.17	2.16	1.54	1.28	1.57
Phe	4.33	3.60	4.69	5.28	5.21	3.71
Thr	3.80	3.33	3.45	4.15	4.02	3.63
Val	4.76	4.37	4.57	4.78	4.98	4.29
Ammonia, % of total N	5.1	9.0	—	—	—	—
Total free AA N, ⁵ % of total N	20.8	23.7	—	—	—	—
NPN, % of total N	58.4	64.9	—	—	—	—
pH	4.7	3.6	—	—	—	—

¹AS = alfalfa silage; CS = corn silage; ESBM = expeller soybean meal; HMSC = high-moisture shelled corn; SSBM = solvent soybean meal.

²NDIN = neutral detergent insoluble N.

³Fraction B3 = NDIN (% of total N) – ADIN (% of total N) (Sniffen et al., 1992).

⁴NFC = 100 – (%NDF – NDIN × 6.25) – %CP – %Fat – %Ash, using tabular values for fat content of these ingredients (NRC, 2001).

⁵Total free AA N = total free AA, mmol × (40.3 mg of N/mmol of total free AA) (Broderick, 1987).

were vortexed and allowed to stand for 30 min at room temperature before filtering through Whatman no. 1 filter paper. Filtrates were stored at -20°C until MUN analysis by an automated colorimetric assay (Broderick and Clayton, 1997) adapted to flow-injection (Lachat Quik-Chem 8000 FIA). Concentrations and yields of fat, true protein, lactose, and SNF, and MUN concentration were all computed as the weighted means from a.m. and p.m. milk yields on each test day. Yield of 3.5% FCM (Sklan et al., 1992) also was computed. Efficiency of conversion of feed DM was calculated for each cow over the last 2 wk of each period by dividing mean yield of actual milk and FCM by mean DM intake. Similarly, apparent efficiency of utilization of feed N (assuming no retention or mobilization of body N) was calculated for each cow by dividing mean milk N output (milk true protein/6.38) by mean N intake. For computation of BW change, BW was measured on 3 consecutive days at the beginning of the experiment and at the end of each period.

Fecal grab samples were collected from all cows approximately 6 h prefeeding (0400 h) and 6 h postfeeding (1600 h) on d 26 or 27 of each period, transferred to aluminum pans, and held at 60°C in a forced-air oven until completely dry. Fecal samples then were ground to pass a 1-mm Wiley mill screen, and a single

composite was prepared for each cow in each period by mixing equal DM from both samples. Fecal samples were analyzed for DM, OM, NDF, ADF, total N, and indigestible ADF as described earlier. Indigestible ADF was used as an internal marker to estimate apparent nutrient digestibility and fecal N output (Cochran et al., 1986). When fecal sampling was done, spot urine samples were obtained from all cows by mechanical stimulation of the vulva. After collection, 15 mL of urine was pipetted into specimen containers holding 60 mL of 0.072 *N* H_2SO_4 and stored at -20°C until analysis. After thawing at room temperature, urine samples were analyzed for creatinine using a picric acid assay (Oser, 1965) adapted to flow-injection analysis (Lachat Quik-Chem 8000 FIA), for total N (Leco FP-2000 Nitrogen Analyzer), and for urea with the colorimetric method used for MUN. Daily urine volume and excretion of urea N and total N were estimated from urinary creatinine concentration assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

Statistical Analysis

The lactation trial was conducted as an incomplete 8×8 Latin square, replicated 6 times. It was intended from the outset to run the trial for 3 periods only,

Table 2. Composition of diets A to H containing 2 levels of CP (15.8 and 17.1%), 2 levels of expeller soybean meal (ESBM; 0 and 5%), and 2 levels of rumen-protected Met¹ (RPM; 0 and 9 g/d)

Item	A	B	C	D	E	F	G	H
	15.8% CP				17.1% CP			
	0% ESBM		5.0% ESBM		0% ESBM		5.0% ESBM	
	RPM: 0	RPM: 9	RPM: 0	RPM: 9	RPM: 0	RPM: 9	RPM: 0	RPM: 9
	% of DM							
Alfalfa silage	20.7	20.7	20.7	20.7	20.7	20.7	20.7	20.7
Corn silage	33.9	33.9	33.9	34.0	33.9	33.9	33.9	33.9
Rolled high-moisture shelled corn	26.0	26.0	25.3	25.3	23.1	23.1	22.4	22.4
Solvent soybean meal	10.0	10.0	5.7	5.7	12.9	12.9	8.6	8.6
Expeller soybean meal ²	0.0	0.0	5.0	5.0	0.0	0.0	5.0	5.0
Soyhulls	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Fat supplement ³	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Ground shelled corn	2.13	2.07	2.13	2.07	2.13	2.07	2.13	2.07
Mepron ⁴	0	0.06	0	0.06	0	0.06	0	0.06
Sodium bicarbonate	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51
Dicalcium phosphate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Limestone	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Dynamate	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Vitamin-trace mineral mix ⁵	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Chemical composition								
CP, % DM	15.8	15.8	15.8	15.8	17.1	17.1	17.1	17.1
Ash, % of DM	5.91	6.14	5.91	6.14	6.09	6.32	6.09	6.32
NDF, % of DM	27.2	27.2	27.6	27.6	27.2	27.1	27.6	27.6
ADF, % of DM	16.4	16.4	16.6	16.6	16.5	16.5	16.6	16.6
NDIN, % of total N	12.6	12.6	15.7	15.6	12.1	12.1	14.9	14.9
ADIN, % of total N	3.6	3.6	4.1	4.0	3.4	3.4	3.8	3.8
Fraction B3, ⁶ % of CP	9.0	9.0	11.6	11.5	8.7	8.7	11.1	11.1
Fat, % of DM	3.4	3.3	3.4	3.8	3.1	3.6	4.0	3.6
NFC, ⁷ % of DM	48.1	47.9	47.7	47.0	46.9	46.2	45.6	45.7
Starch, % of DM	23.0	24.5	26.1	27.9	24.8	24.6	26.3	26.5
RDP, ⁸ % of DM	10.4	10.4	9.8	9.8	11.1	11.1	10.5	10.5
RUP, ⁸ % of DM	5.3	5.4	6.0	6.0	6.0	6.0	6.6	6.6
NE _L , ⁸ Mcal/kg of DM	1.62	1.60	1.63	1.62	1.60	1.61	1.62	1.61

¹Computed assuming 60% metabolizability of RPM in Mepron (Berthiaume et al., 2001) and 24 kg/d of DM intake.

²SoyPlus, West Central Coop (Ralston, IA).

³Energy Booster 100, MS Specialty Nutrition (Dundee, IL).

⁴Rumen-protected Met product from Degussa Corp. (Kennesaw, GA).

⁵Provided (per kg of DM): 56 mg of Zn, 46 mg of Mn, 22 mg of Fe, 12 mg of Cu, 0.9 mg of I, 0.4 mg of Co, 0.3 mg of Se, 6,440 IU of vitamin A, 2,000 IU of vitamin D, and 16 IU of vitamin E.

⁶Fraction B3 = neutral detergent insoluble N (NDIN) (% of total N) – ADIN (% of total N) (Sniffen et al., 1992).

⁷NFC = 100 – (%NDF – NDIN × 6.25) – %CP – %Fat – %Ash.

⁸Estimated using the NRC (2001) model based on observed DM intakes.

rather than for a full 8 periods to avoid confounding effects due to the decline in production that would have occurred later in the lactation curve. The 8 diets were designated A through H (Table 2). Six incomplete 8 × 8 Latin squares were constructed by fixing the first column of treatments, ordered A through H, as the diets fed in period 1, and then randomly assigning 2 of 7 possible columns of treatments (ordered B, C, . . . , A; C, D, . . . , B; . . . ; H, A, . . . , G) as the subsequent series of diets in periods 2 and 3. In this arrangement, columns represented periods and rows represented cows within squares. The 6 groups of cows were blocked as

described and randomly assigned to 1 of these 6 incomplete Latin squares. Using this approach, 40 of the 56 possible changeovers from each diet to every other diet occurred twice and 16 occurred once. Mean intake and production data from the last 14 d of each period, and average excretion and digestibility data obtained using spot fecal and urine sampling at the end of each period were computed for each cow and analyzed as a replicated, incomplete 8 × 8 Latin square using the mixed model of SAS (SAS Institute, 1999–2000). The model included cow (within square), square, period, dietary CP, RUP, and RPM, 7 carryover effects, interactions

of CP $\times$ RUP, CP $\times$ RPM, and RUP $\times$ RPM, as well as the 3-way interaction CP $\times$ RUP $\times$ RPM; all terms were considered fixed except cow (within square), which was considered random. The test for carryover effects, which was included because of the unbalanced design, was analogous to that of Haber and Lucas (<http://www4.stat.ncsu.edu/%7Egumpertz/ggdata/carburetoexample.sas>). Carryover effects in the model were limited to 7 df and may be considered as differences from the mean carryover. Overall, interactions among main effects were not significant ($P \geq 0.09$). Of the carryover effects, 10 out of 203 were significant ($P \leq 0.05$); 8 of these involved changeovers from diets F and H (17.1% CP plus RPM, without or with RUP; Table 2). This indicated that carryover effects did not bias interpretation of results. To assess whether RPM addition to 15.8% CP diets gave production comparable to that on 17.1% CP without RPM (diets A and C vs. diets F and H, Table 2), a mixed model similar to that used for the overall analysis without carryover effects but including “contrast” and “estimate” statements was used to determine response differences and associated 95% confidence intervals on those diets. Interaction terms were removed from both models when $P \geq 0.25$. For all statistical analyses, significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Composition of the major ingredients fed in this study is in Table 1. Corn silage was similar to values in NRC (2001) tables, containing 40% NDF, 23% ADF, and about 8% CP. The chemical composition of the alfalfa silage—nearly 24% CP, 38% NDF and 28% ADF—indicated that it was of very high quality. Both silages had in excess of 50% of total N as NPN, and low levels of ammonia-N and ADIN, indicating that they were well fermented and that N utilization would be expected to be normal. The high-moisture corn, solvent and expeller soybean meals, and soyhulls were also similar in composition to values reported in NRC (2001) tables. Contents of NDF and intake-adjusted NE_L in the 8 diets averaged 27.4% and 1.61 Mcal/kg (Table 2), making them appropriate for high-producing dairy cows. As expected, NFC decreased from a mean of 47.7% to mean of 46.1% when CP was increased by substitution of high-moisture corn by soybean meal. Dietary starch levels, determined on TMR composites, ranged from 23 to 28% of DM. Although analytical results were variable, mean starch levels may be considered in the normal range for lactating cows (Oba and Allen, 2003).

Least squares means from the 8 individual treatments are reported in Table 3. However, there were no

significant interactions ($P \geq 0.09$), so our discussion will concentrate on the main effects of dietary CP and supplementation with RUP and RPM. Higher dietary CP was associated with increased ($P \leq 0.05$) DM intake, milk content of fat and true protein, and yield of milk, 3.5% FCM, fat, true protein, lactose, and SNF (Table 4). Kalscheur et al. (1999) found that increasing dietary CP increased the yields of milk, fat, and protein from wk 4 through 14 of lactation, but not after wk 19 of lactation, in high-producing dairy cows. The present findings paralleled earlier observations of greater production of milk and milk components, on diets formulated from similar feedstuffs, when CP was increased from 15.4 to 16.7% but not from 16.7 to 18.4% CP (Broderick, 2003) and from 15.7 to 16.7% but not from 16.7 to 17.7% CP (Olmos Colmenero and Broderick, 2006b). As expected, increasing dietary CP elevated apparent N digestibility because of greater dilution of metabolic fecal N (Schneider and Flatt, 1975). However, higher dietary CP also improved ($P \leq 0.05$) apparent digestibility of DM, OM, NDF, and ADF. This suggested that RDP might have been inadequate on the lower CP diet. Although no ruminal sampling was done in the present study, ammonia N averaged 6.1 and 7.9 mg/dL in the rumen on a previous trial when diets contained, respectively, 16.1 and 17.3% CP, and total-tract digestibility of organic matter and fiber was linearly related to ruminal ammonia and dietary CP concentration (Broderick et al., 2008). It is not known whether improved digestibility resulted from ruminal or postruminal effects of greater dietary CP. Milk/DM intake and FCM/DM intake were not altered, suggesting that increased dietary CP stimulated production via greater intake and improved digestibility.

Elevated ($P < 0.01$) MUN, urinary excretion of urea N and total N, urea N as a proportion of total urinary N, and total manure N excretion, plus depressed ($P < 0.01$) N efficiency all indicated that utilization of the additional N on the higher CP diet was impaired (Table 4). Several studies have shown that reducing dietary CP improved apparent N efficiency (Wu and Satter, 2000; Broderick, 2003; Olmos Colmenero and Broderick, 2006a), but maximal N efficiency in another study came at the expense of depressed yield of milk and milk components (Broderick et al., 2008). Based on intakes observed in the present trial, the NRC (2001) model predicted NE_L -limited milk yields of 41 and 43 kg/d but MP-limited milk yields of 38 and 43 kg/d for diets containing, respectively, 15.8 and 17.1% CP. This suggested that supply of MP, rather than NE_L , limited production. Moreover, BW gain was similar at about 0.4 kg/d across all diets. The 2 kg/d difference for NE_L -limited milk was the result of the 1.1 kg/d greater DM intake on 17.1% CP. Thus, at equal NE_L intake,

Table 3. Least squares means for production, excretion, and apparent digestibility in lactating dairy cows by dietary treatment

Item	15.8% CP				17.1% CP				SE ³
	0% RUP ¹		5% RUP		0% RUP		5% RUP		
	RPM ² : 0	RPM: 9	RPM: 0	RPM: 9	RPM: 0	RPM: 9	RPM: 0	RPM: 9	
DM intake, kg/d	24.3	24.7	23.9	24.6	25.3	25.9	24.8	25.9	0.53
N intake, g/d	611	616	613	625	707	705	676	698	14.2
BW gain, kg/d	0.38	0.33	0.26	0.44	0.35	0.40	0.52	0.50	0.155
Milk yield, kg/d	39.7	39.4	39.4	41.5	41.0	41.8	41.2	42.9	1.02
Milk/DM intake	1.63	1.60	1.66	1.70	1.63	1.61	1.67	1.68	0.034
3.5% FCM, kg/d	39.7	38.2	40.2	44.2	42.0	43.1	41.9	44.2	1.20
FCM/DM intake	1.64	1.55	1.69	1.80	1.67	1.67	1.69	1.71	0.041
Milk fat, %	3.45	3.45	3.57	3.81	3.65	3.68	3.64	3.79	0.113
Milk fat, kg/d	1.38	1.33	1.42	1.60	1.50	1.54	1.49	1.59	0.055
Milk true protein, %	3.22	3.25	3.13	3.16	3.19	3.29	3.17	3.17	0.050
Milk true protein, kg/d	1.29	1.24	1.24	1.34	1.30	1.36	1.31	1.34	0.032
Milk lactose, %	4.85	4.74	4.83	4.77	4.82	4.83	4.74	4.83	0.052
Milk lactose, kg/d	1.95	1.83	1.93	2.01	1.97	2.02	1.94	2.05	0.055
Milk SNF, %	8.96	8.85	8.84	8.86	8.91	9.02	8.82	8.92	0.094
Milk SNF, kg/d	3.61	3.41	3.53	3.73	3.65	3.76	3.61	3.77	0.093
MUN, mg/dL	9.7	9.0	10.2	10.2	11.2	11.6	11.6	12.1	0.33
Milk-N/N intake, %	32.7	31.3	32.3	34.1	29.6	30.4	30.0	29.8	0.64
Excretion ⁴									
Urine volume, L/d	19.4	19.7	25.7	19.9	20.7	20.4	23.2	24.0	1.85
Urine urea-N, g/d	123	130	131	137	173	173	181	180	5.5
Total urine-N, g/d	178	175	180	176	204	217	214	220	7.2
Urea-N/Total urine-N, %	72.9	74.8	75.2	77.4	86.3	79.7	85.2	83.9	2.77
Fecal DM, kg/d	8.8	9.6	9.1	9.8	9.1	10.1	9.5	8.7	0.34
Fecal N, g/d	236	245	251	266	246	283	269	240	10.8
Manure-N, g/d	411	420	432	442	450	501	484	461	14.6
N-balance, ⁵ g/d	1	-4	-4	-30	49	-24	3	30	11.8
Apparent digestibility ⁶									
DM, %	64.0	61.0	62.0	59.9	63.9	60.5	61.9	66.3	0.94
OM, %	65.1	62.1	63.3	61.1	65.2	62.3	63.4	67.5	0.94
NDF, %	39.1	33.5	37.1	34.9	38.1	34.4	38.4	42.7	1.40
ADF, %	40.6	35.1	37.5	36.2	40.5	36.7	40.0	43.7	1.38
N, %	61.7	60.2	59.5	57.6	65.1	59.3	60.5	65.6	1.26

¹Dietary content of expeller soybean meal, the source of supplemental RUP.²Computed assuming 60% metabolizability of rumen-protected Met (RPM) in Mepron (Berthiaume et al., 2001) and 24 kg/d of DM intake; Mepron from Degussa Corp. (Kennesaw, GA).³Standard error of the least squares means.⁴Urinary excretion estimated using creatinine as a volume marker and fecal excretion estimated using indigestible ADF as an internal marker.⁵Nitrogen balance computed assuming milk true protein contains 6.38% N.⁶Apparent digestibilities estimated from spot fecal sampling, using indigestible ADF as an internal marker.

MP-limited milk yield should have been 3 kg/d lower (equivalent to 90 g/d of protein at 3% milk protein content) on diets containing 15.8% CP. Yield on diets containing 17.1% CP was only greater by 1.7 kg/d of milk and 50 g/d of protein.

The positive effects of feeding more RUP as expeller soybean meal (Table 4) were improved ($P = 0.01$) feed efficiency, higher ($P = 0.03$) milk fat content, and greater ($P \leq 0.02$) yield of FCM and fat. Greater apparent NDF digestibility ($P = 0.04$) was also observed. Heat processing of soybean meal results in formation of protein-bound NDF; NDF-bound CP in feedstuffs often remains digestible in the rumen (Haugen et al., 2006) and total tract (Aldrich et al., 1995). Although milk true protein yield was not altered, RUP supplementa-

tion elevated MUN ($P < 0.01$) and urinary excretion of urea N ($P = 0.01$) and reduced milk protein content ($P = 0.01$). This suggested that the low Met content in soybean meal RUP limited its utilization.

Supplementing with RPM increased ($P \leq 0.05$) feed intake and yield of FCM and fat and gave trends for increased milk fat content and yield of milk and milk true protein (Table 4). Although several studies found greater fat yield with increased Met supply (Munneke et al., 1991; Overton et al., 1996), others reported no effect on this trait (Yang et al., 1986; Pisulewski et al., 1996). Overton et al. (1998) observed contradictory responses with RPM: increased fat yield on a diet high in ground shelled corn but decreased fat secretion on a higher NDF diet containing corn gluten feed. We found

Table 4. Effect of dietary CP content and supplementation of RUP as expeller soybean meal and rumen-protected Met (RPM) on production, excretion, and apparent digestibility in lactating dairy cows

Item	CP, % of DM			RUP, ¹ % of DM			RPM, ² g/d			SE ³
	15.8	17.1	<i>P</i> > F ⁴	0	5	<i>P</i> > F	0	9	<i>P</i> > F	
DM intake, kg/d	24.4	25.5	<0.01	25.1	24.8	0.45	24.6	25.3	0.05	0.37
N intake, g/d	616	696	<0.01	660	653	0.46	652	661	0.31	9.7
BW gain, kg/d	0.35	0.45	0.40	0.37	0.43	0.58	0.38	0.42	0.71	0.077
Milk yield, kg/d	40.0	41.7	<0.01	40.5	41.3	0.24	40.4	41.4	0.10	0.72
Milk/DM intake	1.65	1.65	0.90	1.62	1.68	<0.01	1.65	1.65	0.93	0.026
3.5% FCM, kg/d	40.6	42.8	<0.01	40.8	42.6	0.02	41.0	42.4	0.05	0.83
FCM/DM intake	1.67	1.68	0.55	1.63	1.72	<0.01	1.67	1.68	0.68	0.026
Milk fat, %	3.57	3.69	0.05	3.56	3.70	0.03	3.58	3.68	0.10	0.086
Milk fat, kg/d	1.43	1.53	<0.01	1.44	1.52	0.01	1.45	1.51	0.04	0.039
Milk true protein, %	3.19	3.20	0.63	3.24	3.16	0.01	3.18	3.22	0.16	0.035
Milk true protein, kg/d	1.28	1.33	0.01	1.30	1.31	0.69	1.28	1.32	0.09	0.020
Milk lactose, %	4.80	4.81	0.83	4.81	4.79	0.64	4.81	4.79	0.62	0.033
Milk lactose, kg/d	1.93	2.00	0.05	1.94	1.98	0.31	1.95	1.98	0.41	0.036
Milk SNF, %	8.88	8.92	0.48	8.93	8.86	0.24	8.88	8.91	0.59	0.058
Milk SNF, kg/d	3.57	3.70	0.02	3.60	3.66	0.39	3.60	3.67	0.27	0.059
MUN, mg/dL	9.8	11.6	<0.01	10.4	11.0	<0.01	10.7	10.7	0.80	0.22
Milk-N/N intake, %	32.6	30.0	<0.01	31.0	31.6	0.22	31.2	31.4	0.53	0.38
Excretion ⁵										
Urine volume, L/d	21.2	22.1	0.45	20.1	23.2	0.02	22.2	21.0	0.34	1.05
Urine urea-N, g/d	130	176	<0.01	150	157	0.05	152	155	0.40	3.4
Total urine-N, g/d	177	214	<0.01	194	198	0.40	194	197	0.52	4.0
Urea-N/total urine-N, %	75.1	83.8	<0.01	78.4	80.4	0.31	79.9	78.9	0.63	1.40
Fecal DM, kg/d	9.4	9.4	1.00	9.4	9.3	0.64	9.1	9.6	0.06	0.20
Fecal N, g/d	249	259	0.13	252	256	0.59	250	258	0.26	6.5
Manure-N, g/d	426	474	<0.01	445	455	0.37	444	456	0.24	8.7
N-balance, ⁶ g/d	-9.4	14.8	<0.01	5.7	-0.2	0.49	12.2	-6.7	0.03	5.96
Apparent digestibility ⁷										
DM, %	61.7	63.1	0.03	62.4	62.5	0.81	63.0	61.9	0.11	0.52
OM, %	62.9	64.6	0.01	63.7	63.8	0.83	64.2	63.2	0.13	0.52
NDF, %	36.2	38.4	0.02	36.3	38.3	0.05	38.2	36.4	0.07	0.74
ADF, %	37.4	40.2	<0.01	38.2	39.4	0.25	39.7	37.9	0.08	0.75
N, %	59.7	62.7	<0.01	61.6	60.8	0.38	61.7	60.7	0.23	0.71

¹Dietary content of expeller soybean meal, the source of supplemental RUP.²Computed assuming 60% metabolizability of RPM in Mepron (Berthiaume et al., 2001) and 24 kg/d of DM intake; Mepron from Degussa Corp. (Kennesaw, GA).³Standard error of the least squares means.⁴Probability of the effects of dietary CP content or supplementation with RUP and RPM from Mepron.⁵Urinary excretion estimated using creatinine as a volume marker and fecal excretion estimated using indigestible ADF as an internal marker.⁶Nitrogen balance computed assuming milk true protein contains 6.38% N.⁷Apparent digestibilities estimated from spot fecal sampling, using indigestible ADF as an internal marker.

that RPM supplementation improved production of fat and FCM in 1 of 2 previous trials (Broderick et al., 2008). Feeding RPM has also been shown to increase milk concentrations of total protein (Armentano et al., 1997; Berthiaume et al., 2006), true protein (Berthiaume et al., 2006; Broderick et al., 2008), and casein (Overton et al., 1998), and yields of milk (Schmidt et al., 1999), total protein (Armentano et al., 1997), and true protein (Rulquin and Delaby, 1997).

We were interested in whether RPM supplementation would allow similar production at lower dietary CP and, thus, reduce N excretion. An analysis of results obtained feeding 15.8% CP plus RPM versus 17.1% CP without RPM is in Table 5. This comparison suggested that DM intake and yield of milk and milk components

were not different ($P \geq 0.16$) on diets containing 15.8% CP plus RPM than on diets containing 17.1% CP minus RPM. Also, there was substantially reduced ($P < 0.01$) MUN, urinary excretion of urea N and total N, and proportion of urea in total urinary N, plus more than a 3-percentage-unit improvement ($P < 0.01$) in apparent N efficiency. The only exception noted was that estimated N-balance (computed using true rather than total protein secretion) as well as our relatively low apparent N digestibilities (Table 4) were 44 g/d more negative ($P < 0.01$), suggesting that N may have been mobilized from the tissues on the 15.8% CP diets supplemented with RPM.

Flows of MP and digested (metabolizable) Lys and Met, computed using the NRC (2001) model, were esti-

Table 5. Production and excretion results in lactating dairy cows fed 15.8% dietary CP plus supplemental rumen-protected Met (RPM; 9 g/d) versus 17.1% dietary CP without RPM (0 g/d)

Item	15.8% CP	17.1% CP	Difference	95% Confidence interval		SED ²	<i>P</i> > <i>F</i>
	RPM ¹ : 9	RPM: 0		Lower	Upper		
DM intake, kg/d	24.7	25.3	−0.6	−1.4	0.2	0.4	0.17
N intake, g/d	621	696	−75	−97	−52	11	<0.01
BW gain, kg/d	0.40	0.43	−0.03	−0.34	0.27	0.15	0.82
Milk yield, kg/d	40.3	41.5	−1.2	−2.8	0.5	0.8	0.16
Milk/DM intake	1.65	1.65	0.00	−0.05	0.04	0.02	0.85
3.5% FCM, kg/d	41.3	42.2	−0.9	−2.8	1.1	1.0	0.38
FCM/DM intake	1.68	1.68	0.00	−0.07	0.07	0.04	1.00
Milk fat, %	3.66	3.64	0.02	−0.13	0.17	0.08	0.78
Milk fat, kg/d	1.47	1.50	−0.03	−0.11	0.06	0.04	0.55
Milk protein, %	3.22	3.21	0.03	−0.04	0.11	0.04	0.37
Milk true protein, kg/d	1.29	1.31	−0.02	−0.07	0.03	0.03	0.43
Milk lactose, %	4.76	4.78	−0.02	−0.10	0.07	0.04	0.70
Milk lactose, kg/d	1.92	1.97	−0.04	−0.13	0.05	0.05	0.34
Milk SNF, %	8.86	8.85	0.01	−0.15	0.16	0.08	0.94
Milk SNF, kg/d	3.57	3.64	−0.08	−0.23	0.08	0.08	0.34
MUN, mg/dL	9.7	11.4	−1.7	−2.3	−1.2	0.3	<0.01
Milk-N/N intake, %	32.6	29.8	2.9	1.8	4.0	0.6	<0.01
Excretion ³							
Urine volume, L/d	19.7	22.2	−2.5	−5.9	0.9	1.7	0.15
Urine urea-N, g/d	135	177	−42	−51	−33	5	<0.01
Total urine-N, g/d	177	209	−32	−45	−19	7	<0.01
Urea-N/total urine-N, %	75.9	86.1	−10.1	−15.6	−4.7	2.7	<0.01
Fecal DM, kg/d	9.8	9.3	0.5	−0.1	1.0	0.3	0.12
Fecal N, g/d	257	259	−2	−21	18	10	0.86
N-balance, ⁴ g/d	−17	27	−44	−68	−21	12	<0.01

¹Rumen-protected Met computed assuming 60% metabolizability of RPM in Mepron (Berthiaume et al., 2001) and 24 kg/d of DM intake; Mepron from Degussa Corp. (Kennesaw, GA).

²Standard error of the difference.

³Urinary excretion estimated using creatinine as a volume marker and fecal excretion estimated using indigestible ADF as an internal marker.

⁴Nitrogen balance computed assuming milk true protein contains 6.38% N.

mated to be, respectively, 276, 16, and 4 g/d greater on 17.1 versus 15.8% dietary CP (Table 6). Mean protein yield accounted for only 41 g/d of the extra MP, a 15% rather than 67% recovery (NRC, 2001), possibly indicating that an essential AA rather than total protein limited protein secretion. Kalscheur et al. (2006) recovered 19% of supplemental CP, added to the diet as RDP and RUP from soybean meal, as secreted milk protein. Assuming 2.7% Met and 1.3% Cys in milk true protein (NRC, 1982), we computed a recovery of RPM in milk Met equal to 11% and a recovery of RPM-S in milk Met and Cys of 18%.

Pisulewski et al. (1996) observed a linear response in secretion of milk true protein when infusing Met abomasally to provide up to an estimated 2.4% of protein truly digested in the intestine, in supplement of Lys at 7.3% of protein truly digested in the intestine. The NRC (2001) used breakpoint analyses to estimate Met and Lys requirements for maximal milk protein concentration and yield of, respectively, about 2.4 and 7.2% of MP. These concentrations in the MP may be used to compute the proportions of “requirements” for these 2 essential AA that were met by diets fed in this study (Table 6). The Lys supply computed using the NRC

(2001) model ranged from 89 to 91% of estimated requirement; Met supply averaged 75% of this requirement without RPM and 87% with RPM addition. Feeding RPM also gave comparable improvement in Met status at both protein levels: RPM supplementation increased Met supply from 76 to 87% of estimated requirement at 15.8% CP and from 74 to 86% of estimated requirement at 17.1% CP. This may explain why comparable responses to RPM have been observed at differing concentrations of dietary protein (Leonardi et al., 2003). The RPM-supplemented diets, with estimated Lys:Met ratios of about 3.1, may have had a more favorable AA pattern than the diets without RPM, with estimated Lys: Met ratio of about 3.6 (Table 6). These computations support the argument that Met supply limited production in this experiment.

CONCLUSIONS

A feeding study was conducted to assess the effects of supplementing with RPM in cows fed diets based on alfalfa and corn silages plus high moisture corn, and with CP and RUP varied by addition of soybean meal. Increasing dietary CP from 15.8 to 17.1% improved yield

Table 6. Estimated supply of MP and digested (metabolizable) AA on diets containing 15.8 or 17.1% CP that were supplemented with RUP as expeller soybean meal and rumen-protected Met (RPM)

Item	CP, % of DM		RUP, ¹ % of DM		RPM, ² g/d	
	15.8	17.1	0	5.0	0	9
DM intake, kg/d	24.4	25.5	25.1	24.8	24.6	25.3
Estimated supply ³						
MP, g/d	2,594	2,870	2,690	2,773	2,696	2,768
Digested Lys, g/d	169	185	175	179	175	179
Digested Met, g/d	51	55	53	53	49	58
Digested Lys, % of MP	6.5	6.4	6.5	6.4	6.5	6.5
Digested Met, % of MP	2.0	1.9	2.0	1.9	1.8	2.1
Lys/Met ratio	3.3	3.4	3.3	3.4	3.6	3.1
Estimated requirement ⁴						
Lys requirement, g/d	187	207	194	199	194	199
Met requirement, g/d	63	69	65	67	65	67
Lys supply, % of requirement	90	90	91	89	90	90
Met supply, % of requirement	82	80	82	79	75	87

¹Dietary content of expeller soybean meal, the source of supplemental RUP.

²Rumen-protected Met computed assuming 60% metabolizability of RPM in Mepron (Berthiaume et al., 2001) and 24 kg/d of DM intake; Mepron from Degussa Corp. (Kennesaw, GA).

³Metabolizable protein and digested (metabolizable) Met and Lys estimated using the NRC (2001) model based on observed DM intakes.

⁴Computed assuming digested (absorbed) Lys and Met required at, respectively, 7.2 and 2.4% of MP.

of milk and milk components, primarily via increased intake and digestibility, but also elevated urinary N excretion and depressed apparent N efficiency. Feeding additional RUP as expeller soybean meal improved feed efficiency and fat yield but did not alter N utilization, possibly because of inadequate supply of Met in this source of RUP. Adding RPM to the diet increased yield of FCM and fat, and tended to increase yield of true protein.

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